

In the Specification

Please replace the paragraph beginning on page 1 at line 10 with the following amended paragraph:

Nuclear factor κ B (NF- κ B) is a family of inducible eukaryotic transcription factor complexes participating in regulation of immune response, cell growth, and survival [Ghosh et al. 1998]. The NF- κ B factors are normally sequestered in the cytoplasmic compartment by physical association with a family of cytoplasmic ankyrin rich inhibitors termed I κ B, including I κ B α and related proteins [Baldwin et al. 1996]. In response to diverse stimuli, including cytokines, mitogens, and certain viral gene products, I κ B is rapidly phosphorylated at serines 32 and 36, ubiquitinated and then degraded by the 26S proteasome, which allows the liberated NF- κ B to translocate to the nucleus and participate in target gene transactivation [Mercurio et al 1999, Pahl et al 1999]. Recent molecular cloning studies have identified a multi subunit I κ B kinase (IKK) that mediates the signal-induced phosphorylation of I κ B. The IKK is composed of two catalytic subunits, IKK α and IKK β , and a regulatory subunit IKK γ . The catalytic activity of both IKK α and IKK β can be activated by a multitude of different NF- κ B inducers, including the inflammatory cytokines, tumor necrosis factor and

interleukin-1, the T cell receptor and the T cell costimulatory protein, CD28 [Karin et al 2000].

Please replace the paragraph beginning on page 3 at line 16 with the following amended paragraph:

Assessment of the pattern of the NF- κ B species in lymphoid organs of *aly/aly* mice indicated that, apart from its role in the regulation of NF- κ B complex(s) comprised of Rel proteins (A+p50) and I κ B, NIK also participates in controlling the expression/activation of other NF- κ B species. Most notably, the lymphocytes of the *aly/aly* mice were deficient of p52, an NF- κ B species that is specifically formed in mature B-lymphocytes through proteolytic processing of an inactive precursor, p100 (NF- κ B2), suggesting a deficiency in p100 - p52 conversion [Yamada et al. 2000]. Indeed, NIK has been shown to participate in site specific phosphorylation of p100. ~~Both both directly and through phosphorylation and through phosphorylation~~ of IKK α , which in turn phosphorylates p100. This phosphorylation serves as a molecular trigger for ubiquitination and active processing of p100 to form p52. This p100 processing activity was found to be ablated by the *aly* mutation [Xiao et al. 2001, Senftleben et al. 2001].

Please replace the paragraph beginning on page 8 at line 1 with the following amended paragraph:

Mouse and human IL2 both cause proliferation of T-cells of the homologous species at high efficiency. Human IL2 also stimulates proliferation of mouse T-cells at similar

concentrations, whereas mouse IL2 stimulates human T-cells at a lower (sixfold to 170-fold) efficiency. The involvement of IL-2 in autoimmunity is controversial (reviewed by O'Shea et al. 2002) It is recognized that IL-2 administration is associated with a variety of autoimmune disorders such as immune thyroiditis, rheumatoid arthritis and other ~~arthropaties~~ arthropathies. However IL-2 deficient mice produce multiple autoantibodies, including anti-DNA antibodies. About half die of autoimmune haemolytic anemia and the survivors develop inflammatory bowel disease. Importantly, the pathology is corrected by the addition of exogenous IL-2. This indicates a role of IL-2 in maintaining peripheral tolerance.

Please replace the paragraph beginning on page 8 at line 11 with the following amended paragraph:

IL2 is a growth factor for all subpopulations of T-lymphocytes. The IL2R-alpha receptor subunit is expressed in adult T-cell leukemia (ATL). Since freshly isolated leukemic cells also secrete IL2 and respond to it, IL2 may function as an autocrine growth modulator for these cells capable of worsening ATL.

Please replace the paragraph beginning on page 9 at line 16 with the following amended paragraph:

X-linked severe combined immunodeficiency (XSCID) is a rare and potentially fatal disease caused by mutations of IL2R γ chain, the gene encoding the IL-2R γ chain, a component of multiple cytokine receptors that are essential for lymphocyte development and function (Noguchi et al. 1993). To date, over 100 different mutations of IL2RG resulting in XSCID have been published. Recent gene knock out studies indicate a pivotal role of ~~the γ chain~~ this gene in lymphopoiesis [DiSanto et al 1995].

Please replace the paragraph beginning on page 10 at line 1 with the following amended paragraph:

The present invention relates to the use of IL-2 common gamma chain (c γ c) (SEQ ID NO: 22) or a mutein, variant, fusion protein, preferably 41MDD (SEQ ID NO:2), 44MPD (SEQ ID NO:17), the intracellular domain of c γ c (ICDc γ c) (SEQ ID NO:1), 1-357 (SEQ ID NO:20) 1-341 (SEQ ID NO:21, functional derivative, circularly permuted derivative or fragment thereof for modulating the interaction between c γ c and NIK.

Please replace the paragraph beginning on page 10 at line 8 with the following amended paragraph:

In addition the ~~invention~~ invention relates to the use of a DNA encoding c γ c or a mutein, variant, fusion protein, circularly permuted derivative or fragment thereof, a DNA

encoding the antisense of *cyc*, an antibody specific to *cyc*, or a small molecule obtainable by screening products of combinatorial chemistry in a luciferase system, for modulating the interaction between IL-2 common gamma chain (*cyc*) and NIK.

Please replace the paragraph beginning on page 17 at line 15 with the following amended paragraph:

Figure 11 shows the amino acid sequence of the intracellular domain of *cyc* (SEQ ID NO: 1).

Please replace the paragraph beginning on page 17 at line 16 with the following amended paragraph:

Figure 12 shows the amino acid sequence of the 41 amino acid polypeptide from the membrane distal domain of *cyc* (41MDD) (SEQ ID NO: 2).

Please replace the paragraph beginning on page 17 at line 20 with the following amended paragraph:

Figure 13 shows the nucleotide sequence of the intracellular domain of *cyc* (*cyc*ICD) (SEQ ID NO: 5).

Please replace the paragraph beginning on page 17 at line 22 with the following amended paragraph:

Figure 14 shows the nucleotide sequence of the 41 polypeptide from the membrane distal domain of *cyc* (41MDD) (SEQ ID NO: 6).

Please replace the paragraph beginning on page 17 at line 25 with the following amended paragraph:

Figure 15 shows the sequence of 12 ~~aminoacids~~ amino acids at the C-terminus of *cyc* involved in binding NIK (SEQ ID NO: 3).

Please replace the paragraph beginning on page 18 at line 21 with the following amended paragraph:

Cyc and NIK interaction was detected using a C-terminal fragment of NIK (624-947) as bait in a two-hybrid screen of a bone marrow cDNA library. This interaction was confirmed by co-immunoprecipitation studies carried out in lysates of mammalian cells overexpressing NIK and *cyc* and also by co-immunoprecipitation studies in cells naturally expressing NIK and *cyc*. Immunoprecipitation studies revealed that *cyc* (SEQ ID NO: 22) is efficiently co-precipitated with either the C-terminus of NIK (624-947) or with the full length of NIK.

Please replace the paragraph beginning on page 19 at line 4 with the following amended paragraph:

Multiple deletion mutants of both *cyc* and NIK were generated to define the binding domains in both proteins. The interactions were tested by yeast 2 hybrid tests and/or by immunoprecipitation studies (see examples below). Domains of *cyc* responsible for binding NIK were found in the membrane proximal domain (MPD) of *cyc* comprising 44 amino acid residues (from residue 282 to 325 of SEQ ID NO: 22), named 44MPD (see SEQ ID NO: 17) and, ~~a~~in a membrane distal domain (MDD) comprising 41 amino acid (from residues 329 to 369 of SEQ ID NO: 22), named 41MDD (see SEQ ID NO: 2 and Figure 12). When 12 amino acids at the end of *cyc* (*cyc* residues 358-369, Fig 15 SEQ ID: NO 3 nucleotide sequence in SEQ ID NO: 4) were deleted from the intracellular domain of *cyc* (*cyc*ICD), the binding to NIK decreases by 50% indicating that these residues play a major role in binding.

Please replace the paragraph beginning on page 19 at line 14 with the following amended paragraph:

In addition, mutagenesis was carried out in residues located within the 41MDD, to define the specific amino acids interacting with NIK. The interaction of proline rich motifs in signaling proteins with their cognate domains is well documented (Kay BK, Williamson MP, Sudol M. FASEB J 2000 Feb 14 (2): 231-421). 20% of the amino acids in the membrane distal 41 amino acids of *cyc* are prolines. Therefore, two consecutive prolines

were mutated to alanine at two different sites within the 41 membrane distal amino acids of *cyc*: 1- PP336,337AA (SEQ ID NO: 23) and 2- PP360,361AA (SEQ ID NO: 24) and the effect of the mutation on binding of NIK tested by the two hybrid assay. The results obtained of *cyc* mutagenesis demonstrate that the prolines at residues 360 and 361 are important for the binding to NIK. Thus the muteins of the present invention ~~retains~~ retain prolines at residues 360 and 361.

Please replace the paragraph beginning on page 19 at line 26 with the following amended paragraph:

cyc and NIK interaction was shown to be functionally significant. Reporter gene assays showed that *cyc* modulates NIK-induced NF- κ B activation. It is possible, under experimental conditions, to induce NF- κ B activation by overexpressing NIK. Activation of NF- κ B can be monitored in cells transfected with a construct encoding ~~—lucifrase~~ luciferase under the control of an NF- κ B inducible promoter. Using this luciferase system, NF- κ B activation was monitored in cells overexpressing NIK alone or together with different concentration of *cyc* (for details see examples below). It was found that modulation of NF- κ B depends on the concentration of NIK vis a vis the concentration of *cyc* within the cells (NIK/*cyc*). For example, enhancement of NIK

mediated NF- κ B activation was observed when NIK/cyc was above 1 while inhibition of NIK mediated NF- κ B activation was observed when NIK/cyc was about equal or below 1.

Please replace the paragraph beginning on page 20 at line 26 with the following amended paragraph:

Progressively C-terminus deleted cyc fragments, 1-357, 1-341, 1-325, 1-303, were tested for their ability to modulate NF- κ B mediated by NIK in the luciferase system. For this purpose luciferase expression and activation of NF- κ B was measured in transfected cells overexpressing NIK and cyc or cyc deleted mutants at a ratio of about 1. Under these conditions cyc inhibits NF- κ B activation induced by NIK. It was found that full length cyc (SEQ ID NO: 22) and fragments 1-357 (SEQ ID NO:20), and 1-341 (SEQ ID NO:21) were able to inhibit NIK mediated NF- κ B activation while mutants lacking the NIK binding domain such as 1-325 and 1-303 did not have any effect on the activity of NIK mediated NF- κ B activation. The lack of effect of fragments 1-325 and 1-303 confirms the involvement of the membrane distal domain of cyc-NIK interaction and the role of this interaction in NF- κ B modulation.

Please replace the paragraph beginning on page 22 at line 4 with the following amended paragraph:

The results obtained revealed that signalling ~~through~~ through cyc involves NIK and recruitment of signalosome proteins and consequently modulation of NF- κ B. Therefore cyc or fragments thereof for example those comprising NIK binding domain such as MDD41 or MPD44 (SEQ ID NO:17) could be used to modulate signalling ~~through~~ through cyc

Please replace the paragraph beginning on page 23 at line 13 with the following amended paragraph:

The definition "functional derivatives" as herein used refers to derivatives which can be prepared from the functional groups present on the lateral chains of the amino acid moieties or on the terminal N- or C- groups according to known methods and are comprised in the invention when they are pharmaceutically acceptable i.e. when they do not destroy the protein activity or do not impart toxicity to the pharmaceutical compositions containing them. Such derivatives include for example esters or aliphatic amides of the carboxyl-groups and N-acyl derivatives of free amino groups or O-acyl derivatives of free hydroxyl-groups and are formed with acyl-groups as for example ~~alcanoyl~~ alkanoyl- or aroyl-groups.

Please replace the paragraph beginning on page 33 at line 16 with the following amended paragraph:

A therapeutic or research-associated use of these tools necessitates their introduction into cells of a living organism. For this purpose, it is desired to improve membrane permeability of peptides, proteins and oligonucleotides. Derivatization with lipophilic structures may be used in creating peptides and proteins with enhanced membrane permeability. For instance, the sequence of a known membranotropic peptide as noted above may be added to the sequence of the peptide or protein. Further, the peptide or protein may be derivatized by partly lipophilic structures such as the above-noted hydrocarbon chains, which are substituted with at least one polar or charged group. For example, lauroyl derivatives of peptides have been described by Muranishi et al., 1991. Further modifications of peptides and proteins comprise the oxidation of methionine residues to thereby create sulfoxide groups, as described by Zacharia et al. 1991. Zacharia and co-workers also describe peptide or derivatives wherein the relatively hydrophobic peptide bond is replaced by its ketomethylene isoester—~~(COCH₂)~~(COCH₂). These and other modifications known to the person of skill in the art of protein and peptide chemistry enhance membrane permeability.

Please replace the paragraph beginning on page 44 at line 1 with the following amended paragraph:

The detection of a specific interaction between two different mammalian proteins in a two-hybrid system in yeast does not necessarily imply that there exists a corresponding interaction between the proteins in a native mammalian environment. Therefore, in order to verify NIK and *cyc* interaction in a mammalian environment, co-immunoprecipitation studies of NIK and *cyc* were carried out in lysates of 293-T cells ~~overexpressing~~ overexpressing these proteins (for details see Example 9)

Please replace the paragraph beginning on page 49 at line 13 with the following amended paragraph:

For the generation of the PP336,337AA mutants (SEQ ID NO: 23) the following primers were used:

Please replace the paragraph beginning on page 49 at line 18 with the following amended paragraph:

For the generation of the PP360,361AA mutants (SEQ ID NO: 24) the following primers were used:

Please replace the paragraph beginning on page 49 at line 26 with the following amended paragraph:

For the generation of the K338A mutant (SEQ ID NO: 25)
the following primers were used:

Please replace the paragraph beginning on page 50 at
line 1 with the following amended paragraph:

For the generation of the E344A mutant (SEQ ID NO: 26)
the following primers were used:

Please replace the paragraph beginning on page 50 at
line 5 with the following amended paragraph:

For the generation of the W358A mutant (SEQ ID NO: 27)
the following primers were used

Please replace the paragraph beginning on page 55 at
line 14 with the following amended paragraph:

A cell line was prepared from mouse embryonic
fibroblast cells, which are generally known to express the LT β
receptor. 10^5 cells of the above line were seeded per well in 6
well plates. 24 hours later transfection was performed (with
Gene porter transfection reagent, Gene therapy systems) with the
plasmid pcGST ICcgc expressing the intracellular domain of cyc
(cyc-~~IDC~~ ICD) fused to GST or with pcGST41MDD expressing the 41
distal domain of cyc fused to GST and the expression plasmid
encoding luciferase reporter protein under the control of an NF-

Appln. No. 10/511,722

Amd. dated November 6, 2006

Reply to Office Action of June 25, 2003

κ B inducible promoter (pcDNA3 luciferase). NF- κ B activation was measured indirectly by measuring the luciferase activity present in the cells.